
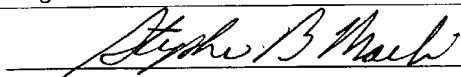


JC10 Rec'd PCT/PTO 28 DEC 2001

FORM PTO-1390 (Modified) (REV 5-93)		U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		ATTORNEY'S DOCKET NUMBER	
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371				065691-0263	
				U.S. APPLICATION NO. 10/019219 Unassigned	
INTERNATIONAL APPLICATION NO. PCT/FR00/01791		INTERNATIONAL FILING DATE 27 June 2000		PRIORITY DATE CLAIMED 28 June 1999	
TITLE OF INVENTION Peptide Compounds Derived From a Shifted Orf of the ICE Gene					
APPLICANT(S) FOR DO/EO/US Christophe Ronsin, Véronique Scott, and Frédéric Triebel					
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:					
1. <input checked="" type="checkbox"/> This is a FIRST submission of items concerning a filing under 35 U.S.C. 371. 2. <input type="checkbox"/> This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371. 3. <input type="checkbox"/> This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1). 4. <input checked="" type="checkbox"/> A proper Demand for International Preliminary Examination was made by the 19 <sup>th</sup> month from the earliest claimed priority date. 5. <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371(c)(2)) <input type="checkbox"/> is transmitted herewith (required only if not transmitted by the International Bureau). <input checked="" type="checkbox"/> has been transmitted by the International Bureau. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US) 6. <input checked="" type="checkbox"/> A translation of the International Application into English (35 U.S.C. 371(c)(2)). 7. <input checked="" type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)) <input type="checkbox"/> are transmitted herewith (required only if not transmitted by the International Bureau). <input type="checkbox"/> have been transmitted by the International Bureau. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired. <input checked="" type="checkbox"/> have not been made and will not be made. 8. <input type="checkbox"/> A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)). 9. <input checked="" type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)). 10. <input checked="" type="checkbox"/> A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)). 11. <input type="checkbox"/> Applicant claims small entity status under 37 CFR 1.27 . Items 12. to 17. below concern other document(s) or information included: 12. <input checked="" type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98. 13. <input checked="" type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included. 14. <input checked="" type="checkbox"/> A FIRST preliminary amendment. <input type="checkbox"/> A SECOND or SUBSEQUENT preliminary amendment. 15. <input type="checkbox"/> A substitute specification. 16. <input type="checkbox"/> A change of power of attorney and/or address letter. 17. <input checked="" type="checkbox"/> Other items or information: Application Data Sheet					

U.S. APPLICATION NO. If known, see 37 CFR 1.50 Unassigned <b>10/019219</b>		INTERNATIONAL APPLICATION NO PCT/FR00/01791		ATTORNEY'S DOCKET NUMBER 065691-0263	
18. <input checked="" type="checkbox"/> The following fees are submitted:				<b>CALCULATIONS</b>	
Basic National Fee (37 CFR 1.492(a)(1)-(5): Search Report has been prepared by the EPO or JPO.....\$890.00					
International preliminary examination fee paid to USPTO (37 CFR 1.482).....\$710.00					
No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) .....\$740.00					
Neither international preliminary examination fee (37 CFR 1.482) nor International search fee (37 CFR 1.445(a)(2)) paid to USPTO ..... \$1,040.00					
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) .....\$100.00					
ENTER APPROPRIATE BASIC FEE AMOUNT =				\$890.00	
Surcharge of \$130.00 for furnishing the oath or declaration later than 20 Months from the earliest claimed priority date (37 CFR 1.492(e))					
Claims	Number Filed		Included in Basic Fee	Extra Claims	Rate
Total Claims	36	-	20	= 16	\$18.00
Independent Claims	2	-	3	= 0	\$84.00
Multiple dependent claim(s) (if applicable)					\$280.00
TOTAL OF ABOVE CALCULATIONS =					\$1178.00
Reduction by 1/2 for filing by small entity, if applicable.					\$0.00
SUBTOTAL =					\$1178.00
Processing fee of \$130.00 for furnishing English translation later the 20 months from the earliest claimed priority date (37 CFR 1.492(f). +					
TOTAL NATIONAL FEE =					\$1178.00
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property +					\$40.00
TOTAL FEES ENCLOSED =					\$1218.00
					Amount to be: refunded \$
					charged \$
a. <input checked="" type="checkbox"/> A check in the amount of \$1218.00 to cover the above fees is enclosed. b. <input type="checkbox"/> Please charge my Deposit Account No. <u>19-0741</u> in the amount of \$0.00 to the above fees. A duplicate copy of this sheet is enclosed. c. <input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. <u>19-0741</u> . A duplicate copy of this sheet is enclosed.					
NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.					
SEND ALL CORRESPONDENCE TO:  <b>FOLEY &amp; LARDNER</b> Customer Number: 22428  <b>22428</b> <small>PATENT TRADEMARK OFFICE</small> Telephone: (202) 672-5407 Facsimile: (202) 672-5399			 SIGNATURE  NAME <u>STEPHEN B. MAEBIUS</u>  REGISTRATION NUMBER <u>35,264</u>		

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Applicants: Christophe Ronsin et al.

Title: Peptide Compounds Derived From A Shifted ORF of the iCE Gene

Serial No.: Unassigned

Filing Date: December 28, 2001

**PRELIMINARY AMENDMENT**

Commissioner for Patents  
Box PCT  
Washington, D.C. 20231

Sir:

Prior to examination of the present application, Applicants respectfully request that the above-identified application be amended as follows:

**In the Claims:**

In accordance with 37 C.F.R. § 1.121(c) (3), please substitute for pending claims 3, 6, 9, 11, 13-31 with the following clean version of the claims. The changes to these claims are shown explicitly in the attached "Marked Up Version of Claims."

3. (Once Amended) A peptide compound as claimed in claim 1, characterized in that it comprises at least one element other than natural amino acids.

6. (Once Amended) A method for revealing artificial point modifications or mutations which are capable of increasing the immunogenicity of the peptide compounds as claimed in claim 1, characterized in that it comprises the following steps:

- a) Determining fragments which possess a sequence of approximately 9 to 10 amino acids comprising an anchoring motif for a given HLA molecule,
- b) introducing an additional point modification (for example a post-

translational modification) or mutation at residues 4, 5, 6, 7 or 8,

c) determining the immunogenicity of the peptide fragments obtained in step b), preferably by carrying out an Elispot assay.

9. (Once Amended) A DNA fragment encoding at least one peptide fragment of claim 1.

11. (Once Amended) A vector for expressing a peptide fragment, characterized in that said fragment comprises a sequence of at least 8 consecutive amino acids of the sequence SEQ ID No. 1, containing a DNA fragment of claim 10 fused to a promoter which is effective in eukaryotic cells and/or in prokaryotic cells, in particular in human cells.

13. (Once Amended) A vector as claimed in claim 11, characterized in that it is a viral vector, a plasmid or a pseudovector.

14. (Once Amended) A dendritic cell loaded with peptide compounds as claimed in claim 1.

15. (Once Amended) A dendritic cell transformed with the expression vector as claimed in claim 11.

16. (Once Amended) A dendritic cell as claimed in claim 14, characterized in that it forms part of the macrophages.

17. (Once Amended) A pharmaceutical composition comprising a peptide compound or a mixture of peptide compounds as claimed in claim 1 and a pharmaceutically acceptable vehicle.

18. (Once Amended) A pharmaceutical composition comprising an expression vector as claimed in claim 11 and a pharmaceutically acceptable vehicle.

19. (Once Amended) A pharmaceutical composition comprising in particular a

DNA fragment as claimed in claim 9 and a pharmaceutically acceptable vehicle.

20. (Once Amended) A pharmaceutical composition comprising the cells as claimed in claim 14 and a pharmaceutically acceptable vehicle.

21. (Once Amended) A pharmaceutical composition as claimed in claim 17, characterized in that it also comprises one or more immunological adjuvants, in particular agents which are cytotoxic for tumors.

22. (Once Amended) A pharmaceutical composition as claimed in claim 17, characterized in that it comprises a pharmaceutical vehicle which is compatible with IV, subcutaneous, oral or nasal administration.

23. (Once Amended) A pharmaceutical composition as claimed in claim 17, characterized in that it comprises a pharmaceutical vehicle selected from positively or negatively charged liposomes, nanoparticles or lipid emulsions.

24. (Once Amended) Use of a peptide compound as claimed in claim 1 for manufacturing a medicinal product.

25. (Once Amended) Use of a peptide compound as claimed in claim 1 for manufacturing a medicinal product intended for treating cancer.

26. (Once Amended) Use of a peptide compound as claimed in claim 1 for manufacturing a medicinal product intended for immunization ex vivo, which consists in particular in inducing tumor-specific CTLs in vitro, expanding them and reinjecting them, said induction possibly being carried out with the aid of loaded dendritic cells.

27. (Once Amended) Use of a peptide compound as claimed in claim 1 for manufacturing a medicinal product intended for immunization in vivo.

28. (Once Amended) Use of a peptide compound as claimed in claim 1 for manufacturing a medicinal product intended for the treatment of cancer, in particular solid tumors, especially carcinomas, melanomas, neuroblastomas, preferably hepatocarcinomas

and adenocarcinomas of the colon or of the kidney.

29. (Once Amended) Use of a peptide compound as claimed in claim 1 for increasing, in culture medium, the CTL population of tumors and/or inducing the secretion by said CTLs of cytotoxic factors such as, for example, IL-2, IFN  $\gamma$  and TNF.

30. (Once Amended) Use of a peptide compound as claimed in claim 1 for manufacturing a medicinal product intended for stimulating immune defenses, in particular to increase the CTL population of tumors and/or to induce the secretion by said CTLs of cytotoxic factors such as, for example, IL-2, IFN- $\gamma$  and TNF.

31. (Once Amended) A method for producing an antibody which recognizes a peptide compound as claimed in claim 1, comprising the steps consisting in:

- a) immunizing a mammal with said peptide compound,
- b) isolating a monoclonal antibody which binds to said peptide in an immunological assay.

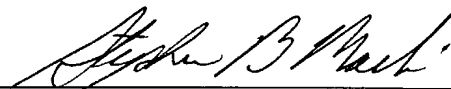
#### REMARKS

Applicants respectfully request that the foregoing amendments be made prior to examination of the present application. The amendments are made to correct multiple dependencies and do not change the scope of the invention.

Respectfully submitted,

Date: December 28, 2001

By



FOLEY & LARDNER  
Customer Number: 22428



22428

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Stephen B. Maebius  
Attorney for Applicants  
Registration No. 35,264

# **MARKED UP VERSION OF AMENDED CLAIMS**

3. (Once Amended) A peptide compound as claimed in [either of claims 1 and 2] claim 1, characterized in that it comprises at least one element other than natural amino acids.

6. (Once Amended) A method for revealing artificial point modifications or mutations which are capable of increasing the immunogenicity of the peptide compounds as claimed in [one of claims 1 to 3 and 5] claim 1, characterized in that it comprises the following steps:

- a) Determining fragments which possess a sequence of approximately 9 to 10 amino acids comprising an anchoring motif for a given HLA molecule,
- b) introducing an additional point modification (for example a post-translational modification) or mutation at residues 4, 5, 6, 7 or 8,
- c) determining the immunogenicity of the peptide fragments obtained in step b), preferably by carrying out an Elispot assay.

9. (Once Amended) A DNA fragment encoding at least one peptide fragment of [one of claims 1 to 3, 5, 7 and 8] claim 1.

11. (Once Amended) A vector for expressing a peptide fragment, characterized in that said fragment comprises a sequence of at least 8 consecutive amino acids of the sequence SEQ ID No. 1, [as claimed in one of [lacuna] 1 to 3, 5, 7 and 8], containing a DNA fragment of claim 10 fused to a promoter which is effective in eukaryotic cells and/or in prokaryotic cells, in particular in human cells.

13. (Once Amended) A vector as claimed in [either of claims 11 and 12] claim 11, characterized in that it is a viral vector, a plasmid or a pseudovector.

14. (Once Amended) A dendritic cell loaded with peptide compounds as claimed in [one of claims 1 to 3, 5, 7 and 8] claim 1.

15. (Once Amended) A dendritic cell transformed with the expression vector as claimed in [one of claims 11 to 13] claim 1.

16. (Once Amended) A dendritic cell as claimed in [either of claims 14 and 15] claim 14, characterized in that it forms part of the macrophages.

17. (Once Amended) A pharmaceutical composition comprising a peptide compound or a mixture of peptide compounds as claimed in [one of claims 1 to 3, 5, 7 and 8] claim 1 and a pharmaceutically acceptable vehicle.

18. (Once Amended) A pharmaceutical composition comprising an expression vector as claimed in [one of claims 11 to 13] claim 11 and a pharmaceutically acceptable vehicle.

19. (Once Amended) A pharmaceutical composition comprising in particular a DNA fragment as claimed in [either of claims 9 and 10] claim 9 and a pharmaceutically acceptable vehicle.

20. (Once Amended) A pharmaceutical composition comprising the cells as claimed in [one of claims 14 to 16] claim 14 and a pharmaceutically acceptable vehicle.

21. (Once Amended) A pharmaceutical composition as claimed in [one of claims 17 to 20] claim 17, characterized in that it also comprises one or more immunological adjuvants, in particular agents which are cytotoxic for tumors.

22. (Once Amended) A pharmaceutical composition as claimed in [one of claims 17 to 21] claim 17, characterized in that it comprises a pharmaceutical vehicle which is compatible with IV, subcutaneous, oral or nasal administration.

23. (Once Amended) A pharmaceutical composition as claimed in [one of claims 17 to 22] claim 17, characterized in that it comprises a pharmaceutical vehicle selected from positively or negatively charged liposomes, nanoparticles or lipid emulsions.

24. (Once Amended) Use of a peptide compound as claimed in [one of claims 1 to



3, 5, 7 and 8] claim 1 for manufacturing a medicinal product.

25. (Once Amended) Use of a peptide compound as claimed in [one of claims 1 to 3, 5, 7 and 8] claim 1 for manufacturing a medicinal product intended for treating cancer.

26. (Once Amended) Use of a peptide compound as claimed in [one of claims 1 to 3, 5, 7 and 8] claim 1 for manufacturing a medicinal product intended for immunization ex vivo, which consists in particular in inducing tumor-specific CTLs in vitro, expanding them and reinjecting them, said induction possibly being carried out with the aid of loaded dendritic cells.

27. (Once Amended) Use of a peptide compound as claimed in [one of claims 1 to 3, 5, 7 and 8] claim 1 for manufacturing a medicinal product intended for immunization in vivo.

28. (Once Amended) Use of a peptide compound as claimed in [one of claims 1 to 3, 5, 7 and 8] claim 1 for manufacturing a medicinal product intended for the treatment of cancer, in particular solid tumors, especially carcinomas, melanomas, neuroblastomas, preferably hepatocarcinomas and adenocarcinomas of the colon or of the kidney.

29. (Once Amended) Use of a peptide compound as claimed in [one of claims 1 to 3, 5, 7 and 8] claim 1 for increasing, in culture medium, the CTL population of tumors and/or inducing the secretion by said CTLs of cytotoxic factors such as, for example, IL-2, IFN  $\gamma$  and TNF.

30. (Once Amended) Use of a peptide compound as claimed in [one of claims 1 to 3, 5, 7 and 8] claim 1 for manufacturing a medicinal product intended for stimulating immune defenses, in particular to increase the CTL population of tumors and/or to induce the secretion by said CTLs of cytotoxic factors such as, for example, IL-2, IFN- $\gamma$  and TNF.

31. (Once Amended) A method for producing an antibody which recognizes a peptide compound as claimed in [one of claims 1 to 3, 5, 7 and 8] claim 1, comprising the steps consisting in:

Atty. Dkt. No. 065691/0263

- a) immunizing a mammal with said peptide compound,
- b) isolating a monoclonal antibody which binds to said peptide in an immunological assay.

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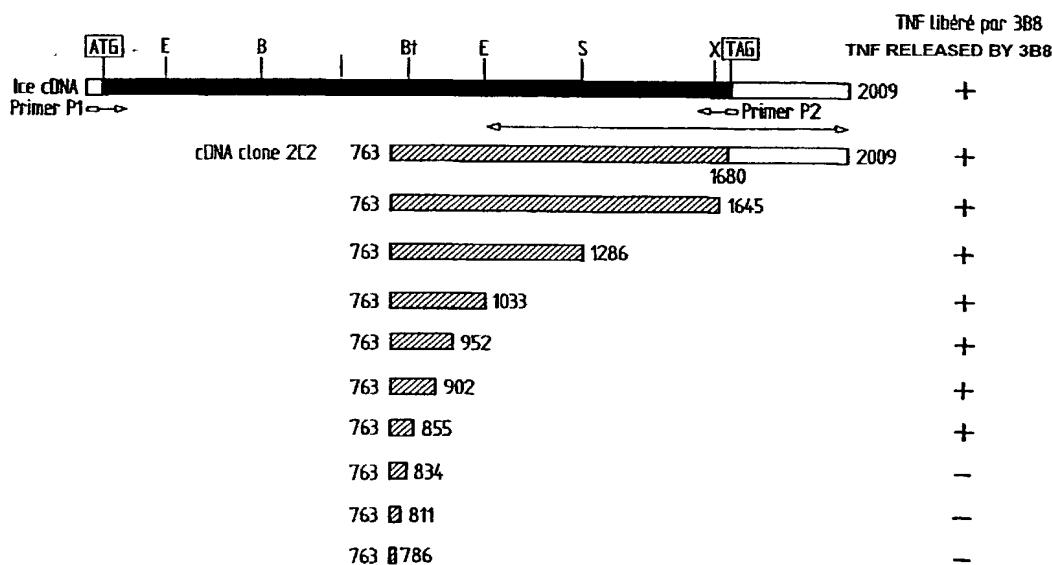
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[Suite sur la page suivante]

(54) Title: COMPOUND DERIVED FROM AN OFFSET ORF OF THE ICE GENE

(54) Titre : COMPOSE PEPTIDIQUE DERIVE D'UNE ORF DECALEE DU GENE ICE



(57) Abstract: The invention concerns a peptide compound, causing a T specific response from tumours, comprising a sequence of at least 8 consecutive amino acids of the peptide sequence coded by a phase offset sequence of the ice gene. The invention also concerns a pharmaceutical composition comprising said peptide compound and the use of said compounds for making a medicine for cancer treatment, in particular for treating solid tumours.

[Suite sur la page suivante]

WO 01/00784 A3

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**PEPTIDE COMPOUND DERIVED FROM A SHIFTED  
ORF OF THE ICE GENE.**

The present invention relates to a peptide compound  
5 which causes a tumor-specific T response, and which  
comprises a sequence of at least 8 consecutive amino  
acids of the peptide sequence encoded by a frame-  
shifted sequence of the ice gene. The invention also  
relates to a pharmaceutical composition comprising said  
10 peptide compound and to the use of these compounds for  
manufacturing a medicinal product intended for treating  
cancer, in particular for treating solid tumors.

Various products turn out to be recognized by T cells  
15 which are reactive with respect to tumors, most of them  
being isolated from patients with melanomas. Some of  
these antigens (Ag) represent products of nonmutated  
genes whose expression in normal adult tissues is  
restricted to the testicles (MAGE-1, MAGE-3, BAGE and  
20 GAGE) (1-4). Other nonmutated genes are differentiation  
antigens which are also expressed, for example, in  
normal melanocytes, but not in other normal tissues.  
These differentiation antigens comprise the melanocyte  
line gene products MART-1/MelanA (5, 6), gp100 (6),  
25 tyrosinase (7, 8) and gp75 (9). T cells which are  
reactive with respect to melanomas also turn out to  
recognize mutated products of the  $\alpha$ -catenin (10), MUM1  
(11) and CDK-4 (12) genes. T cells which are reactive  
with respect to renal cell carcinoma (RCC) also turn  
30 out to recognize products of point-mutated genes such  
as HLA-A2 (13) or HSP70-2 (14).

In addition, some Ags which are recognized by reactive  
T cells can be generated by modified transcription  
35 products comprising intron sequences, as in the case of  
MUM-1 (11), N-acetylglucosaminyltransferase-V (GnT-V)  
(15) or gp100 (16). The T-cell surveillance of cell  
integrity may focus on peptides encoded by an  
alternative open reading frame (ORF) located inside the

- 2 -

primary ORF, as in the case of gp75/TRP-1 (17) and NY-E50-1 (18). Few examples exist in the literature on the use of alternative ORFs in eukaryotes, and the biological significance of the corresponding products is unknown. However, it may be assumed that these products might be used as antigenic targets, and increase the effectiveness of immune surveillance. Specifically, There is an increasingly clear relationship between the abnormal translational control of gene expression (such as for c-mys or FGF-2) (19-21) and the appearance of cancer, and thus the immunogenic peptides in tumors may originate from peptides which derive from the primary ORF, but also from alternative ORFs.

The screening of a cDNA library with a clone of T cells which are reactive with respect to the HLA-B7-restricted renal cell carcinoma (RCC), and which derive from tumor-infiltrating lymphocytes (TILs) which have been amplified by cloning in vivo, led, in the context of the present invention, to the isolation of a nonamer encoded by an alternative (A + 1 frameshift) open reading frame (ORF) of the intestinal carboxylesterase (iCE) gene. This peptide binds to HLA-B\*0702-presenting molecules, as determined in a binding assay by immunofluorescence using transfected T2 cells. The constitutive expression of this alternative-ORF protein was observed in all the transformed HLA-B7\* renal cell lines which were recognized by TILs in cytotoxicity assays. The iCE gene is transcribed in RCC tumors, as well as in normal liver, intestine and kidney tissues. A mutation in the natural ATG translation start site does not impair recognition, which shows that the frameshift (i.e. sliding the ribosome forward) and the recoding are not the mechanisms involved. In addition, a point mutation in the three AUG codons which can be used as alternative translation start sites in the +1 ORF does not abolish recognition, whereas the mutation of an upstream ACG codon does so, indicating that the

- 3 -

latter codon initiates the translation of the alternative ORF. Unexpectedly, this alternative ORF is thus initiated from a non-AUG (ACG) cryptic codon.

## 5 Description

Thus, the present invention relates to a peptide compound which leads to a tumor-specific T response, and which comprises a sequence of at least 8 consecutive amino acids of the peptide sequence encoded by the frame-shifted sequence (A+1 or A+2) of the iCE gene. The nucleotide sequence and peptide sequence of iCE (Homo sapiens intestinal carboxylesterase; liver carboxylesterase-2) are available on the site [www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov) under the access number NM\_3869.

The publication Schwer, H., Langmann, T., Daig, R., Becker, A., Aslanidis, C. and Schmitz, G. Molecular cloning and characterization of a novel putative carboxylesterase, present in human intestine and liver. Biochem. Biophys. Res. Commun. 233 (1), 117-120 (1997) (MEDLINE 97289502) is incorporated in the description by way of reference.

The invention relates more specifically to a peptide compound which causes a specific T response, characterized in that it comprises a sequence of at least 8 consecutive amino acids of the following sequence SEQ ID No. 1:

TVVRLFLAWLPCMMVPCWLPWRTWWWSSSSTAWVSWASSALETSTQPATGATWTK  
30 WLHYAGSSSRISPTLEATLTVSPFLASLRVARVCLRLLCPPYPKDSSTEPSWRVAW  
PSCPASLPAQLMSSPRWWPTCLPVTCLTLRPWWAACGARVKRRFLQLTSLSR.

Mention may be made in particular of a peptide compound which has at least 80% identity with the sequence SPRWWPTCL (SEQ ID No. 2).

The invention also relates to a method for identifying peptide compounds comprising a sequence which has at least 80% identity with a sequence of approximately 9

- 4 -

to 10 consecutive amino acids of the sequence SEQ ID No. 1, characterized in that it comprises the following steps:

- 5 a) Determining fragments which possess a sequence of approximately 9 to 10 amino acids comprising an anchoring motif for a given HLA molecule,
- b) determining the immunogenicity of the peptide fragments obtained in step a), preferably by  
10 carrying out an Elispot assay.

A subject of the invention is the peptide compounds which can be obtained from this method.

15 The peptide fragments to be assayed can be easily obtained by chemical synthesis based on general knowledge in the technical field.

The Elispot assay is widely described in the documents of the prior art. For example, Herr et al, (1998)  
20 relates to an Elispot method for detecting and quantifying CD8 + T lymphocytes which secrete TNF- $\alpha$ . In summary, MultiScreen-HA plates (Millipore, Bedford, MA) are covered with an anti-TNF- $\alpha$  antibody (clone 195; Boehringer Mannheim) and CD8 + T lymphocytes are added  
25 in the presence of antigenic peptides. The secreted TNF- $\alpha$  is detected with a rabbit anti-TNF- $\alpha$  antibody (Serotec, Oxford, UK), a biotin-coupled rabbit anti-IgG antibody (Boehringer Mannheim) and the biotin-avidin-peroxidase complex (Vector, Burlingame, CA). The number  
30 and the surface area of the areas where the cytokine is present are determined automatically by computer, (Herr et al, 1997). Other documents, such as Herr et al, (1996) materials and methods section paragraph 2 pages 132 to 135, and Scheibenbogen et al, (1997) page 933,  
35 describe this method and are also incorporated in the description by way of reference.

In addition, the invention relates to a method for revealing artificial point modifications or mutations

- 5 -

which are capable of increasing the immunogenicity of the peptide compounds described above, said method comprising the following steps:

- 5 a) Determining fragments which possess a sequence of approximately 9 to 10 amino acids comprising an anchoring motif for a given HLA molecule,
- b) introducing an additional point modification (for example a post-translational modification) or  
10 mutation at residues 4, 5, 6, 7 or 8,
- c) determining the immunogenicity of the peptide fragments obtained in step b), preferably by carrying out an Elispot assay.

15 This method is well known to persons skilled in the art. It is possible in particular to incorporate into the description, by way of reference, the teachings which are to be found at the following Internet address:

20 [www.bimas.dcrn.nih.gov/molbio/hla-bind/](http://www.bimas.dcrn.nih.gov/molbio/hla-bind/)

This method makes it possible to determine any artificial (not present in human tumors) point modification or mutation which is thought to be capable  
25 of improving the active principle (the immunogenic mutated peptide), using the so-called "reverse immunology" method. Based on the knowledge of the amino acid sequence of a protein, it is possible to predict which of the peptides are capable of binding to an HLA  
30 pocket regardless of its specificity (HLA-A2, HLA-A1, HLA-B7, etc.), then to test these peptides in vitro for their capacity to effectively bind to the HLA allele under consideration, and then to introduce a point modification or mutation on the amino acids in certain  
35 positions which are critical for affinity. The BIMAS computer program makes it possible to obtain such a prediction. The general rules concerning the amino acids involved in anchoring to HLA molecules are set out in Parker et al, (1992 and 1994) and Rammensee et



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- Protective chemical groups which are known to persons skilled in the art and which react with the NH<sub>2</sub> and/or COOH ends of a peptide, this modification not

- 7 -

significantly decreasing the immunogenic nature of the peptide.

- Chemical groups which improve the effectiveness of the vaccine in vivo.
- 5 - Lipids or fatty acids which are covalently bonded to the peptide fragments so as to form peptide compounds which are termed lipopeptides. Palmitic acid is one example among others, Vitiello et al, (1995), which has been incorporated into the description by way of
- 10 reference.
- A carrier protein for said peptide fragments which possesses restriction sites and enables the intact peptide fragments to be conveyed to their sites of action in the body.

15

Thus, the peptide compound according to the invention can comprise at least one element other than natural amino acids.

- 20 An additional embodiment of the invention relates to a DNA fragment encoding at least one peptide fragment defined above. This fragment can comprise a sequence which has at least 50% identity with a sequence of at least 24 consecutive nucleotides of the following
- 25 sequence SEQ ID No. 3:

acggtggtgcgcttggttttggcatggcttccttgatgatggttccatgctggc  
 tgccttggagaacgtgggtgggtgcatcatccagtagcgcctgggtgtcctgggc  
 ttcttcagcactggagacaagcagcaaccggcaactggggctacctggaccaag  
 30 tggtgacactacgttgggtccagcagaatatcgcccactttggaggcaaccctga  
 ccgtgtcaccatttttggcgagctctgcgggtggcagagtggtgtcttcgcttggt  
 gtgtcccccataatcccaaggactcttccacggagccatcatggagagtggtggtg  
 ccctcctgcccggcctcattgccagctcagctgatgtcatctccacggtgggtggc  
 caacctgtctgctgtgaccaagttgactctgaggccctgggtgggctgctgcgg  
 35 ggcaagagtaaagaggagattcttgcaattaacaagcctttcaagatgatccccg  
 gagtgggtggatgggggtcttcctgccc

This sequence corresponds to the A + 1 alternative ORF of the iCE gene which is expressed in tumor cells. The

- 8 -

expression product of this ORF is recognized by a clone of T cells which are reactive with respect to the HLA-B7-restricted RCC. The reactive TILs are amplified in situ in the tumor site.

5

The term "DNA fragments" is intended to mean single-stranded or double-stranded DNA, cDNA and/or RNA fragments. The nucleotide sequence corresponding to the amino acid sequence of said peptide fragments can vary so as to comprise all the various possible codons for a given amino acid according to the principle of degeneracy of the genetic code. A subject of the present invention is also a vector for expressing a peptide fragment, containing an abovementioned DNA fragment fused to a promoter which is strong and effective in eukaryotic cells and/or in prokaryotic cells, in particular in human cells. The vector can be viral, a plasmid vector or a pseudovector, and can comprise selection markers and express immunological adjuvants such as cytokines and/or lymphokines.

The invention also relates to dendritic cells loaded with peptide compounds and dendritic cells transformed with the expression vector expressing the peptide fragments. These cells can also be macrophages. Nestle et al, (1998), describe a vaccination method which consists in loading the dendritic cells taken from a patient with antigenic peptides (in culture in vitro) and injecting them into the lymphatic system of this same patient. This publication is cited in the description by way of reference.

The subject of another aspect of the invention is a pharmaceutical composition comprising a peptide compound or a mixture of peptide compounds according to the invention and a pharmaceutically acceptable vehicle. This composition can also comprise one or more immunological adjuvants, in particular factors which are cytotoxic for tumors.

- 9 -

The invention also relates to a pharmaceutical composition comprising an expression vector as mentioned above and a pharmaceutically acceptable vehicle, or a DNA fragment according to the invention,  
 5 or alternatively the cells indicated above, and a pharmaceutically acceptable vehicle.

The pharmaceutical composition or the combination product according to the invention can also comprise  
 10 one or more immunological adjuvants, in particular agents which are cytotoxic for tumors. These products can comprise a pharmaceutical vehicle which is compatible with IV, subcutaneous, oral or nasal administration, and which is preferably selected from  
 15 positively or negatively charged liposomes, nanoparticles or lipid emulsions.

Another aspect of the invention relates to the use of a peptide compound as defined above for manufacturing a  
 20 medicinal product in particular intended for the treatment of cancer, in particular solid tumors, especially carcinomas, melanomas, neuroblastomas, preferably hepatocarcinomas and adenocarcinomas of the colon or of the kidney. This medicinal product may be  
 25 intended for immunization ex vivo, which consists in particular in inducing tumor-specific CTLs in vitro, expanding them and reinjecting them, said induction possibly being carried out with the aid of loaded dendritic cells or with an immunization in vivo. The  
 30 invention also relates to the use of said peptide compound for increasing, in culture medium, the CTL population of tumors and/or inducing the secretion by said CTLs of cytotoxic factors such as, for example, IL-2, IFN- $\gamma$  and TNF, and/or for stimulating immune  
 35 defenses, in particular to increase the CTL population of tumors and/or to induce the secretion by said CTLs of cytotoxic factors such as, for example, IL-2, IFN- $\gamma$  and TNF.

- 10 -

In an additional embodiment, the invention relates to a method for producing an antibody which recognizes a previously described peptide compound, comprising the steps consisting in:

- 5 a) Immunizing a mammal with said peptide compound,
- b) isolating a monoclonal antibody which binds to said peptide in an immunological assay.

10 The invention is also directed toward a monoclonal antibody which can be obtained using this method.

The invention is also directed toward a method for detecting a peptide or polypeptide encoded by the A + 1 ORF of iCE, comprising the steps consisting in:

- 15 a) Bringing a sample removed from an individual into contact with an abovementioned monoclonal antibody,
- b) allowing the formation of the peptide or polypeptide/antibody complex,
- 20 c) detecting said peptide or polypeptide by means of a detectable label which is in the complex or which binds to the complex;

and a diagnostic kit comprising in particular said antibody for detecting cancer, in particular for the prognostic of existing cancer in an individual.

25 A composition comprising in particular said monoclonal antibody and a pharmaceutically acceptable vehicle may also be useful in the context of the cancer treatment.

30 The iCE cDNA was isolated originally from a human small intestine cDNA library (31). It exhibits 65% homology with other carboxylesterases of various mammalian species. It is expressed in human intestine, liver and kidney, and appears to play an important role in xenobiotic control and detoxification of the intestinal mucosa (31). A large series of T-cell epitopes encoded in the minimum nucleotide region of the regular iCE ORF was tested, and none of them were recognized in the context of the class I HLA-B\*0702-restricted element.

35

- 11 -

Conversely, a 453-nt ORF encoded in this region following a +1 frameshift turned out to encode a nonamer with HLA-B7-anchoring residues at positions 2, 3 and 9 (SPRWWPTCL, SEQ ID No. 2). A semi-maximal lysis  
5 was obtained with less than  $10^{-6}$  M of nonapeptide in target sensitization assays. The binding of this nonapeptide to T2 cells transfected with HLA-B\*0702 is stable with time, suggesting that low amounts of expression of this alternative ORF are sufficient to  
10 induce T-cell recognition in vitro and T-cell proliferation in vivo, as shown, in the latter case, by the in situ amplification at the tumor site of the corresponding TIL subpopulation.

15 The results obtained in the context of the invention reveal that a novel mechanism is involved in the generation of T-cell epitopes. An alternative ORF induced by a non-AUG cryptic codon which leads to a +1 translational reading frame has proved to encode a  
20 tumor Ag recognized by TILs. In two other examples, gp75/TRP-1 (17) and NY-E50-1 (18), peptides recognized by TILs are encoded by an alternative ORF located within the primary ORF. A mechanism by which the alternative ORF is translated has been suggested for  
25 gp75/TRP-1 (17), for which recognition is affected by the presence of an internal AUG preceding the epitope. In addition to this ribosomal screening mechanism, a ribosomal frameshift (39, 40) has been suggested for the production of T-cell epitopes (41), but, in the  
30 case of the iCE gene, this possibility is excluded since mutating the natural ATG translation start site does not affect peptide recognition. In fact, the presence of the first cryptic internal translation start site (an ACG codon at position 440) in the +1  
35 alternative ORF of iCE is sufficient to direct the expression of sufficient amounts of iCE peptide for the activation of T cells in vitro, as well as in vivo (i.e. leading to clonal expansion of T cells in situ). The leaky screening model, in which ribosomes



- 13 -

ratios. Blocking of the lysis with the mAb W6.32 is also represented.

**(B) Cytotoxicity of 3B8 with respect to various allogenic cell lines.**

5 3B8 was tested with the autologous RCC-1 line and various allogenic RCC cell lines (RCC-3, RCC-4 and RCC-5) in a standard chromium assay at an E:T ratio of 18:1. By way of control, the mAb W6.32 was used to block class I HLA molecules which are involved in  
10 antigen presentation.

**Figure 2: Size analysis of CDR3 in TILs and in clone 3B8 using selected primers TCRBV (A) and TCRBJ (B).**

The RNA was subjected to reverse transcription and  
15 amplification over 40 cycles using the primers TCRBV5 and BC. The DNA obtained was copied over 5 cycles in an elongation reaction using the nested fluorescent primer TCRBC (A) or TCRBJ152 (B) (13 BJ primers tested, BJ1S1-BJ1S7, BJ2S1-BJ2S6). The amplified products were  
20 analyzed on an automated sequencer. The profiles obtained show the sizes in nt (x axis) and the intensity of fluorescence (y axis) of the amplified products. The absolute FU values obtained for the dominant peaks are indicated.

25

**Figure 3: Stimulation of CTL clone 3B8 by COS-7 cells transiently cotransfected with the expression vector pcDNAI containing the 2C2 or 3G7 cDNA clone and the autologous HLA-B\*0702 cDNA.**

30 The control stimulating cells comprise the RCC-1 cell line, which is used as a positive control, and COS-7 cells transfected with the HLA-B\*0702 cDNA alone, which are used as a negative control. The iCE cDNA was transiently cotransfected into COS-7 cells with the  
35 HLA-B\*0702 cDNA, and clone 3B8 was added after 48 hours. TNF production was determined by its cytotoxic effect on WEHI cells, 18 hours later. The control stimulating cells comprise the RCC-1 cell line, which is used as a positive control, and COS-7 cells



- 14 -

transfected with HLA-B\*0702 alone, which are used as a negative control.

**Figure 4: Location of the iCE cDNA sequence encoding the antigenic peptide recognized by 3B8.**

This is a schematic representation of the full length iCE cDNA sequence, of the cDNA 2C2 clone and of various truncated 2C2 cDNAs. The untranslated 5' and 3' regions are represented by outlined boxes. The translated sequence of human iCE is represented by a filled-in box; the cDNA clone 2C2 is represented by a dotted box and the truncated 2C2 cDNAs are indicated by hatched boxes. The nucleotides are numbered starting from the natural ATG nonsense codon. The small black frames with an arrow indicate the position of the P1 primer and of the P2 primer. The cDNA used as a probe for hybridizing the RNA transfer is indicated by a two-tipped arrow. Restriction sites (B: Bam HI; Bs: BstX I; E: EcoR I; S: Sma I; X: Xba I). The recognition, by CTL clone 3B8, of COS-7 cells transiently transfected with the autologous HLA-B\*0702 cDNA and with various truncated cDNAs is indicated. The transfected cells were incubated for 24 hours with 5000 3B8 cells, and the amount of TNF in the supernatants was measured via the cytotoxicity effect on WEHI-13 cells.

**Figure 5: Lysis, by CTL clone 3B8, of autologous EBV-transformed cell lines incubated with the iCE encoded peptide.**

2000 EBV-transformed cells were incubated and labeled with <sup>51</sup>Cr for 1 h in the presence of the HLA-B7-restricted iCE peptide or another control HLA-B7-restricted peptide. Clone 3B8 was then added as an effector, in a ratio set at 30:1. Chromium release was measured after 4 h.

**Figure 6: Induction of HLA-B7 expression on T2 cells by the iCE peptide.**



# **Example 1: Materials and methods**

## **Cell lines**

K562 cells were cultured, and the B cell line  
 5 originating from patient 1 which was transformed by EBV  
 in medium consisting of RPMI (Gibco-BRL, Paisley, GB)  
 supplemented with 1% of 200 mM L-glutamine, 1% of  
 200 mM sodium pyruvate, 1% of Hepes, 5% of fetal calf  
 serum (FCS) and 50 IU/ml of penicillin (Gibco-BRL,  
 10 Paisley, GB) was obtained. WEHI-164 clone 13 (W13) and  
 COS-7 cells were cultured in RPMI (Seromed, Biochrom  
 KG, Berlin) supplemented with 1% of 200 mM L-glutamine,  
 1% of 200 mM sodium pyruvate, 1% of Hepes, 5% of fetal  
 calf serum (FCS) and 50 IU/ml of penicillin.

15

## **Patients and establishment of RCC cell lines**

The RCC cell lines were established as previously  
 described (22). Primary tumors were obtained from  
 untreated patients who had undergone a radical  
 20 nephrectomy. The RCC-1 cell line was established from  
 patient 1 (HLA A1, A32, B7, B12-44, Cw5, Cw7), this  
 patient being a 56-year-old man with a clear and  
 granular renal cell carcinoma without metastases. After  
 surgery, fragments were treated by enzymatic digestion,  
 25 and the tumor cell suspensions were cultured in  
 complete RCC medium (22). The RCC-2 (HLA A1, A3, B7,  
 B8, Cw7, Cw7), RCC-3 (HLA A1, A29, B22, B15-62/63, Cw1,  
 Cw7-17), RCC-4 (HLA A3, A19-29, B7, B12-44, Cw7, Cw16),  
 RCC-5 (HLA A1, A3, B6, B22-56, Cw1, Cw7), RCC-6 (HLA  
 30 A9-24, A32, B12-44, B18, Cw5, Cw5), RCC-7 (HLA A1, A28-  
 68, B8, B40-60, Cw3, Cw7) and RCC-8 (HLA A2, A10-25,  
 B18, B13, Cw8, Cw6) tumor cell lines which derive from  
 the primary tumor of patients 2, 3, 4, 5, 6, 7 and 8,  
 respectively, were maintained in complete RCC medium.

35

## **Generation of CTLs from TILs of patient 1**

Autologous TILs were generated from a thawed suspension  
 of dissociated tumor cells. An autologous mixed  
 lymphocyte/tumor cell culture (MLTC) was prepared as

follows: on day 1, dissociated tumor cells were seeded in a proportion of  $2 \times 10^6$  TILs in 6-well flat-bottomed plates (Falcon, Becton Dickinson, New Jersey) in RPMI 1640 (Gibco-BRL, Paisley, GB) containing 1% of 200 mM L-glutamine, 1% of 200 mM sodium pyruvate, 8% of human AB serum (Institut Jacques Boy, S.A., Reims, France) and 50 IU/ml of penicillin, supplemented with 5% of T-cell growth factor (TCGF) and 50 IU/ml of human interleukin-2 (rIL-2) (Roussel Uclaf, Romainville, France), hereafter termed "MLTC complete medium". The MLTC complete medium was discarded every three days as required, and was replaced with fresh MLTC complete medium. On days 7, 15 and 21,  $2 \times 10^6$  TILs were restimulated with  $2 \times 10^5$  irradiated (100 Gray) autologous tumor cells seeded in 6-well flat-bottomed plates with MLTC complete medium. On day 15, the cytotoxic activity of the TILs was tested against the autologous RCC-1 and K562 cell lines, the surface phenotype was characterized by direct immunofluorescence and the cells were cloned by the limiting dilution technique. The TILs were seeded in a proportion of 0.6 to 600 cells/well in V-shaped 96-microwell plates (Nunc, Denmark) which had been seeded beforehand with irradiated autologous tumor cells ( $1 \times 10^4$ /well) as stimulators, and irradiated allogenic PBLs ( $8 \times 10^4$ /well) and irradiated EBV-transformed B cells ( $2 \times 10^4$ /well) as feeder cells, in a total volume of 200  $\mu$ l of MLTC complete medium. Every 3 days, 60  $\mu$ l of supernatant was removed from each well and replaced with 60  $\mu$ l of fresh medium. The cytotoxicity of the clones was determined in a 4-h standard chromium release assay. Every 7-10 days, CTL clones were restimulated with the allogenic feeder cell line and the autologous tumor cell line, as described above.

#### Cytotoxicity assay

The cytolytic activity of the CTLs was determined in a standard  $^{51}\text{Cr}$  release assay as previously described

- 18 -

(22). Target cells (RCC and K562 cell lines) were labeled for 1 h with 50  $\mu$ Ci to 100  $\mu$ Ci of  $^{51}\text{Cr}$  (Du Pont, NEN, Boston, MA) at 37°C, and  $2 \times 10^3$  cells were seeded in 96-microwell plates in 100  $\mu$ l of RPMI supplemented with 5% of FCS. Effector cells were added to the wells at various E:T ratios ranging from 40:1 to 0.1:1. For the lysis inhibition with mAbs, target cells were preincubated for 2 h in the presence of a saturating concentration of mAb before adding the effector cells. The 96-microwell plates were incubated at 37°C for 4 h, and  $^{51}\text{Cr}$  release was determined in the harvested supernatants. For the blocking of cytotoxicity or the production of TNF, the following mAbs were used: W6/32, which is a pan-class I MHC mAb, and B1.23.2 (ME1), which is an HLA-B/C-specific mAb.

#### **Transfection of COS-7 cells and screening of transfection products**

Transfection experiments were carried out with COS-7 cells using the DEAE/dextran/chloroquin method (5, 7, 23). Three days before transfection, COS-7 cells were seeded in 96-microwell flat-bottomed plates, in a proportion of  $5 \times 10^3$  cells/well, in 150  $\mu$ l of RPMI containing 20% of FCS. The transfection experiments were carried out in duplicate in two different microwell plates. For the transfection, the medium was discarded and then replaced with 30  $\mu$ l of transfection mixture containing 35  $\mu$ g of DEAE/dextran (Sigma) and 0.1 mM of chloroquin (Sigma), with 100 ng of plasmid DNA representing a group of approximately 200 recombined clones originating from the cDNA library and 100 ng of the autologous HLA-B\*0702 plasmid. The COS-7 cells were incubated for 4 h at 37°C, and then the medium was removed and the cells were incubated for 2 minutes in a 1x PBS buffer containing 10% of dimethylsulfoxide solution. The cells were washed once in 1x PBS buffer, and were incubated with RPMI containing 10% of FCS for 2 days. After 2 days, the ability of the transfected COS-7 cells to stimulate TNF

- 19 -

production by clone 3B8 was tested, as determined by the WEHI assay.

The ability of the transfected COS-7 cells to stimulate  
 5 TNF production was tested (24).  $2 \times 10^3$  CTLs (clone 3B8)  
 were added to 96-microwell flat-bottomed plates  
 containing transiently transfected COS-7 cells in  
 100  $\mu$ l of RPMI containing 10% of FCS. 18 h later, each  
 supernatant was harvested, and its TNF content was  
 10 determined by assaying its cytotoxic effect on WEHI-164  
 clone 13 cells (25) in a colorimetric assay with  
 3-[4,5-dimethylthiozol]-2,5-diphenyltetrazolium bromide  
 (MTT).

#### 15 **CDR3 size analysis**

The size analysis of CDR3 of TCRBV gene segments which  
 are expressed by the CTL clone 3B8, or which are found  
 in blood or tumor fragments, was carried out as  
 previously described (22). The procedure used for the  
 20 CDR3 size analysis comprises independent RT-PCR  
 amplifications of TCRBV-BC fragments (26), followed by  
 a "flow" of the PCR products using TCRBC or TCRBJ  
 fluorescent nested primers (27) and determination of  
 fluorescent flow product size by electrophoresis on an  
 25 ABI 373 automated DNA sequencer (Applied Biosystems,  
 Inc. Foster City, CA) using the Immunoscope program  
 (28). Since the 5' and 3' primer positions are fixed,  
 variations in size of the flow products are due only to  
 differences in length of the CDR3 regions. Each peak is  
 30 characterized by its position (CDR3 size) and an  
 intensity of fluorescence (arbitrary fluorescence units  
 or FU). The diagrams representing CDR3 size motifs are  
 calibrated at 100% for the highest peaks. In blood  
 originating from healthy donors, most of the profiles  
 35 which reflect CDR3 size diversity in a given  $V\beta$   
 subfamily exhibited 5 to 8 peaks 3 nucleotides apart,  
 with an almost Gaussian distribution (21). The dominant  
 peaks were defined as being very strong signals, with a  
 considerable decrease in the other CDR3 signals.

### **Construction of the cDNA library**

The poly(A)+ RNA was extracted from the RCC-1 cell line using a Maxi Message Marker® kit (R&D Systems, Abingdon, GB), following the manufacturer's instructions. The first strand cDNA was synthesized with the Superscript Choice System® (Gibco BRL, Gaithersburg, MD) using an oligo-dT primer containing a Not I site at its 5' end, and then the second strand cDNA was synthesized. Semi-Bst XI linkers (InVitrogen) were ligated onto the blunt end of the cDNAs, and then digested with Not I and fractionated by chromatography on Sephacryl S-500 HR columns. cDNA fractions were subcloned into the Bst XI and Not I sites of the expression vector pcDNAI. The recombinant plasmids were subjected to electrophoresis in E.coli MC1061/P3, and the bacteria were selected on LB agar plates with 50 µg/ml of ampicillin and 10 µg/ml of tetracycline. In the screening experiments, the RCC-1 cDNA library was divided into 400 groups of 200 cDNA clones. Each group of bacteria was amplified, and the plasmid DNA was extracted using the alkaline lysis method (29).

### **Isolation of the full length iCE cDNA and of iCE cDNAs which are mutated by point mutation or truncated.**

The total RNA was extracted from an RCC cell line using the guanidine isothiocyanate/cesium chloride centrifugation procedure (30). A reverse transcription was carried out on 5 µg of total RNA in a 20 µl reaction volume using the cDNA Cycle® kit, following the manufacturer's instructions. 1 µl of the cDNA reaction mixture was used in a PCR reaction using Taq DNA polymerase (Perkin Elmer). For amplifying human iCE cDNA (31), the following primers were used:

- Primer P1, 5'-CCCAAGCTTGGTGAATAGCAGCGTGTCCGC-3' (nucleotides 28 to 48, sense, SEQ ID No. 4).
- Primer P2, 5'-TGCTCTAGAAGGGAGCTACAGCTCTGTGTG-3' (nucleotides 1666 to 1687, antisense, SEQ ID No. 5).

- 21 -

The conditions for the PCR are as follows: 10 min at 95°C, followed by 30 amplification cycles (94°C for 1 min, 60°C for 2 min, 72°C for 3 min, with a final extension for 10 min at 72°C).

5

The PCR product thus obtained is then digested with Hind III and Xba I and is subcloned into the Hind III and Xba I sites of the expression vector pcDNAI for sequencing. The published sequence of the iCE cDNA carries the access number Y09616. iCE mutants were obtained by site directed mutagenesis by encoding the desired point mutation in overlapping oligonucleotide primers and generating the mutants by PCR (32). Sequencing of the PCR products was carried out with an ABI PRISM DNA sequencing kit (PE Applied Biosystems).

15

#### **Northern blot analysis**

The total RNA was extracted from various primary tumors using a guanidinium isothiocyanate/cesium chloride centrifugation technique (30). The poly(A<sup>+</sup>) RNA was prepared as described above from RCC cell lines and from nontransformed renal cell lines. 5 µg of poly(A<sup>+</sup>) RNA or 10 µg of total RNA were subjected to electrophoresis in a formaldehyde gel containing 1.2% of agarose, and were transferred onto Hybond-N<sup>+</sup> nylon membranes (Amersham, GB). The transferred RNA was hybridized both with a fragment of 2C2 cDNA corresponding to nucleotides 1033 to 2009 of the published human iCE cDNA sequence (31) and with a glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA, as probes. All the probes were labeled with alpha[32P]dCTP (3000 Ci mmol<sup>-1</sup>) using the Prime-IT<sup>TM</sup> II random primer labeling kit (Stratagene, La Jolla, CA). The hybridization was carried out at 48°C for 16 hours.

35

The membranes were washed twice with 2 × SSC at 52°C and once for 15 minutes with 0.2 SSC/0.1% SDS, and then they were autoradiographed or analyzed with a Phosphor-



- 22 -

Imager (Molecular Dynamics, Sunnyvale, California, USA).

**Example 2: An RCC-specific CTL clone was isolated from the TILs**

TILs originating from patient 1 were stimulated with irradiated autologous tumor cells in the presence of a low dose of IL-2 and of TCGF (22). After 15 days of MLTC, specific cytolytic activity against the autologous tumor cells (31% of lysis at an E:T ratio of 40/1) was detected, and TILs were cloned by the limiting dilution technique in the presence of autologous tumor cells, EBV-transformed B cells and allogenic PBLs, and with addition of IL-2 and of TCGF. A TCR $\alpha/\beta^+$  CF8 $^+$  clone, termed 3B8, was isolated. It lyses the autologous RCC cell line, but not the NK-sensitive K562 target cells. The cytotoxicity of clone 3B8 against the autologous RCC-1 cell line, with mAb W6/32, was blocked (Figure 1A). In both the cytotoxicity (Figure 1B) and TNF production assays, all the allogenic HLA-B7 $^+$  RCC cell lines (RCC-2, RCC-4 and RCC-5 in Figure 1B) and none of the HLA-B7 $^-$  RCC cell lines (RCC-7, RCC-6, RCC-7 and RCC-8) are recognized by 3B8. Consequently, the antigen recognized by 3B8 is presented by the HLA-B7 molecule and turns out to be commonly expressed in the RCC cell lines. The 6 class I HLA molecules were isolated from RCC-1 by RT-PCR (33), were cloned into pcDNA1 and were sequenced. The nucleotide sequence of the autologous HLA-B7 cDNA made it possible to identify the allele involved as being HLA-B\*0702. A transfection of this HLA allele into two HLA-B7 $^-$  allogenic RCC cell lines proves sufficient to induce recognition (TNF secretion) by the CTL clone 3B8, confirming the fact that this clone has led to the identification of a shared antigen which is expressed by all RCCs.



extracted from positive duplicates. Finally, a third screening in COS-7 cells led to the isolation of 2 identical cDNA clones (cDNA clones 2C2 and 3G7) which transfer the expression of the antigen into HLA-B7<sup>+</sup> COS-7 cells. The results obtained with these cDNA clones are represented in Figure 3A.

The 2C2 cDNA sequence is 1250 nt long and has 100% homology over nt 763 to 2009 (the nt being numbered starting from the nonsense codon) with a recently identified cDNA which encodes a putative intestinal carboxylesterase (31). In order to identify the full length iCE cDNA corresponding to the published sequence, an RT-PCR was carried out starting from total RNA extracted from an RCC cell line, and the corresponding 1.6-kb PCR product was subcloned into the vector pcDNAI and then sequenced. The nucleotide sequence is identical to the published iCE sequence. Cotransfection experiments in COS-7 cells showed that the full length iCE cDNA is capable of conferring recognition by 3B8.

#### **Example 5: Identification of the antigenic peptide**

In order to delimit the minimum nucleotide region encoding the antigenic peptide, various truncated cDNAs, corresponding to the iCE coding region, were obtained from the 2C2 cDNA clone (Figure 4). These cDNA fragments, which had been subcloned into the expression vector pcDNAI, were transfected into COS-7 cells together with pcDNAI containing the autologous HLA-B\*0702 cDNA. A minimum nucleotide coding region is located between nucleotides 763 and 1033.

In order to reduce the nucleotide sequence encoding the antigen, several truncated cDNAs were obtained by PCR amplification. These truncated cDNAs were cotransfected with the HLA-B\*0702 allele into COS-7 cells. The COS-7 cells transfected with a fragment ranging from nucleotides 763 to 855 are recognized by the CTL clone

- 25 -

3B8, but those transfected with a fragment ranging from nucleotides 763 to 834 (Figure 4) are not, indicating that the peptide coding region is located between nucleotides 763 and 855. After examining the  
5 corresponding amino acid sequence, all possible nonamers and decamers were synthesized, and their ability to make autologous EBV-transformed B cells sensitive to lysis by 3B8 was evaluated. None of them proved to be positive at  $10^{-4}$  or  $10^{-5}$  M.

10 Finally, an alternative ORF was found (a +1 translational open reading frame leading to a 453-nt ORF) with three ATGs in nt positions 476, 479 and 803, which encodes a nonamer (SPRWWPTCL) in the minimum  
15 region of nt 763-855. This nonamer sequence comprises HLA-B7-anchoring residues in positions 2, 3 and 9. Semi-maximum lysis of EBV-transformed B cells was obtained with less than  $10^{-6}$  M of this nonapeptide (Figure 5).

20

#### **Example 6: Binding of the iCE peptide to HLA-B7**

HLA-A2-binding peptide antigens upregulate the expression of HLA-A2 molecules on T2 cells (37). Similarly, T2 cells transfected with HLA-B\*0702 (38)  
25 were used to analyze the binding capacity and the stability of the iCE peptide (Figure 6). At 50 mM, the binding of the iCE peptide is stable over time for at least 4 h, unlike the control, which is the HLA-A2-restricted HSP70 peptide (14).

30

#### **Example 7: Tissue distribution of iCE mRNA**

In order to determine the tissue determination of iCE messengers, a human RNA Master blot<sup>TM</sup> (Clontech, Palo Alto, USA), consisting of a nylon membrane on which  
35 poly(A)+ RNAs originating from 50 human tissues had been immobilized in individual spots, was hybridized with the <sup>32</sup>P-labeled cDNA of clone 2C2, which was used as a probe. The iCE mRNA was detected in the liver, the kidney, the small intestine, the colon and the heart,



still be recognized (Figure 8), indicating that neither the natural amino acid sequence of iCE, nor a chimeric sequence resulting from a programmed translational frameshift (i.e. a sliding of the ribosome in iCE from a codon in a forward direction) and from a recoding of the downstream sequence (39, 40), encodes the recognized peptide.

In addition to the ribosomal frameshift (41), a ribosomal scanning mechanism which initiates translation at a downstream ATG proved to lead to the production of alternative reading frames which are recognized by T cells (42). Point mutations in the full length iCE cDNA were introduced at each of the three ATG sites which were found in the +1 ORF upstream of the nonamer peptide (mutant C for positions 476 and 479, and mutant D at position 803), in order to test whether the corresponding mutated iCE pcDNA1 hybrids were still capable of conferring recognition by CTL clone 3B8 in TNF-release assays, when they were cotransfected with HLA-B\*0702 in COS cells. As shown in Figure 8B, none of these mutations abolishes recognition by CTL clone 3B8. These results demonstrate that a non-AUG cryptic codon is used in the iCE cDNA as an alternative translation start site.

In order to delimit the minimum nucleotide region encoding this non-ATG cryptic codon, stop codons which should interrupt the +1 ORF were introduced at various positions upstream of the antigenic peptide (between positions 428 and 809), with point mutations at positions 466 (mutant E), 519, 666 and 786 of the full length iCE cDNA (Figure 8A). These four mutants abolish all CTL clone 3B8 recognition after cotransfection (see in Figure 8B the result of mutant E for position 446). A minimum nucleotide region was then located between nt 428 and 466. Possible non-ATG codons (CTG, ACG) were then sought in this short sequence, and an ACG codon at position 440 was found. Mutation of this codon into ACT

- 28 -

(mutant F) abolishes CTL clone 3B8 recognition (Figure 8B). Thus, the first non-AUG codon in the +1 ORF was used to initiate the translation process.

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**CLAIMS**

1. A peptide compound, characterized in that it comprises a sequence of at least 8 consecutive amino acids of the sequence SEQ ID No. 1, and in that it causes a specific T response.
2. A peptide compound as claimed in claim 1, characterized in that it comprises a sequence which has at least 80% identity with the sequence SPRWWPTCL (SEQ ID No. 2).
3. A peptide compound as claimed in either of claims 1 and 2, characterized in that it comprises at least one element other than natural amino acids.
4. A method for identifying peptide compounds comprising a sequence which has at least 80% identity with a sequence of approximately 9 to 10 consecutive amino acids of the sequence SEQ ID No. 1, characterized in that it comprises the following steps:
  - a) determining fragments which possess a sequence of approximately 9 to 10 amino acids comprising an anchoring motif for a given HLA molecule,
  - b) determining the immunogenicity of the peptide fragments obtained in step a), preferably by carrying out an Elispot assay.
5. A peptide compound which can be obtained using a method as claimed in claim 4.
6. A method for revealing artificial point modifications or mutations which are capable of increasing the immunogenicity of the peptide compounds as claimed in one of claims 1 to 3 and 5, characterized in that it comprises the following steps:

- 37 -

- a) Determining fragments which possess a sequence of approximately 9 to 10 amino acids comprising an anchoring motif for a given HLA molecule,
  - b) introducing an additional point modification (for example a post-translational modification) or mutation at residues 4, 5, 6, 7 or 8,
  - c) determining the immunogenicity of the peptide fragments obtained in step b), preferably by carrying out an Elispot assay.
7. A peptide compound which can be obtained using a method as claimed in claim 6, characterized in that it comprises a sequence of approximately 9 to 10 amino acids of the sequence SEQ ID No. 1 which has at least one mutation or one modification with respect to the sequence SEQ ID No. 1, and in that it causes a specific T response.
8. A peptide compound as claimed in claim 7, characterized in that it is derived from the sequence SPRWWPTCL (SEQ ID No. 2).
9. A DNA fragment encoding at least one peptide fragment of one of claims 1 to 3, 5, 7 and 8.
10. A DNA fragment as claimed in claim 9, characterized in that it comprises a sequence which has at least 50% identity with a sequence of at least 24 consecutive nucleotides of the sequence SEQ ID No. 3.
11. A vector for expressing a peptide fragment as claimed in one of [lacuna] 1 to 3, 5, 7 and 8, containing a DNA fragment of claim 10 fused to a promoter which is effective in eukaryotic cells and/or in prokaryotic cells, in particular in human cells.



- 38 -

12. An expression vector as claimed in claim 11, also comprising one or more selection marker(s) and, optionally, one or more sequence(s) encoding factors which activate immune defenses, such as cytokines and/or lymphokines.  
5
13. A vector as claimed in either of claims 11 and 12, characterized in that it is a viral vector, a plasmid or a pseudovector.  
10
14. A dendritic cell loaded with peptide compounds as claimed in one of claims 1 to 3, 5, 7 and 8.
15. A dendritic cell transformed with the expression vector as claimed in one of claims 11 to 13.  
15
16. A dendritic cell as claimed in either of claims 14 and 15, characterized in that it forms part of the macrophages.  
20
17. A pharmaceutical composition comprising a peptide compound or a mixture of peptide compounds as claimed in one of claims 1 to 3, 5, 7 and 8 and a pharmaceutically acceptable vehicle.  
25
18. A pharmaceutical composition comprising an expression vector as claimed in one of claims 11 to 13 and a pharmaceutically acceptable vehicle.
- 30 19. A pharmaceutical composition comprising in particular a DNA fragment as claimed in either of claims 9 and 10 and a pharmaceutically acceptable vehicle.
- 35 20. A pharmaceutical composition comprising the cells as claimed in one of claims 14 to 16 and a pharmaceutically acceptable vehicle.



28. Use of a peptide compound as claimed in one of claims 1 to 3, 5, 7 and 8 for manufacturing a medicinal product intended for the treatment of cancer, in particular solid tumors, especially carcinomas, melanomas, neuroblastomas, preferably hepatocarcinomas and adenocarcinomas of the colon or of the kidney.
29. Use of a peptide compound as claimed in one of claims 1 to 3, 5, 7 and 8 for increasing, in culture medium, the CTL population of tumors and/or inducing the secretion by said CTLs of cytotoxic factors such as, for example, IL-2, IFN  $\gamma$  and TNF.
30. Use of a peptide compound as claimed in one of claims 1 to 3, 5, 7 and 8 for manufacturing a medicinal product intended for stimulating immune defenses, in particular to increase the CTL population of tumors and/or to induce the secretion by said CTLs of cytotoxic factors such as, for example, IL-2, IFN- $\gamma$  and TNF.
31. A method for producing an antibody which recognizes a peptide compound as claimed in one of claims 1 to 3, 5, 7 and 8, comprising the steps consisting in:
  - a) immunizing a mammal with said peptide compound,
  - b) isolating a monoclonal antibody which binds to said peptide in an immunological assay.
32. A monoclonal antibody which can be obtained using the method as claimed in claim 31.
33. A method for detecting a peptide or polypeptide encoded by the ORF+1 of iCE, comprising the steps consisting in:



## **ABSTRACT**

The present invention relates to a peptide compound which causes a tumor-specific T response, and which comprises a sequence of at least 8 consecutive amino acids of the peptide sequence encoded by a frame-shifted sequence of the iCE gene. The invention also relates to a pharmaceutical composition comprising said peptide compound and to the use of these compounds for manufacturing a medicinal product intended for treating cancer, in particular for treating solid tumors.

1 / 10

FIG.1A

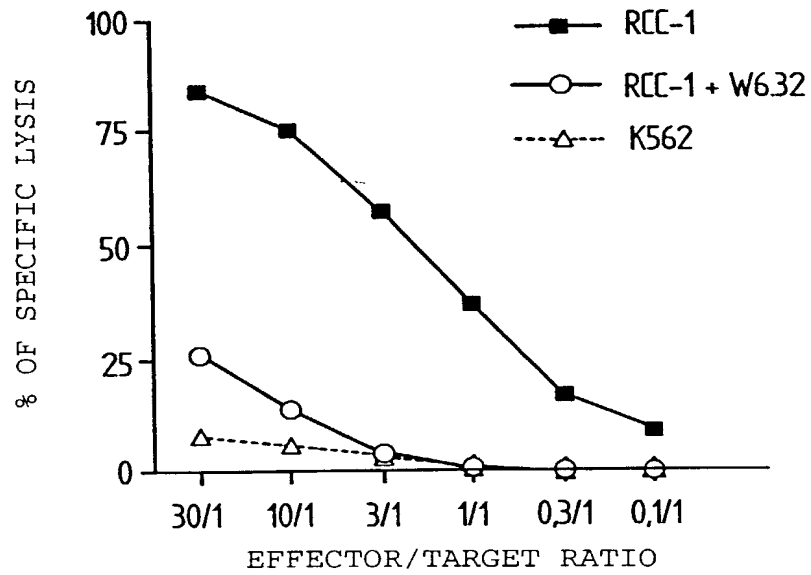
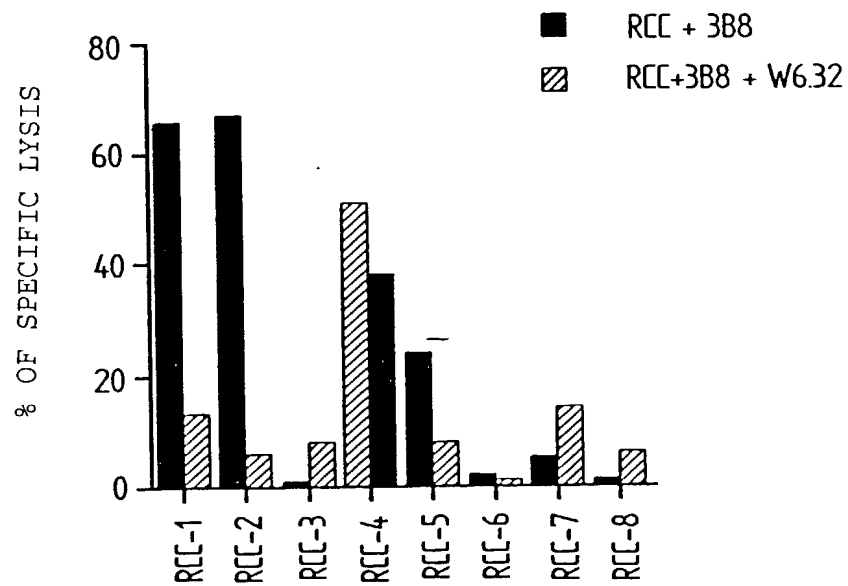


FIG.1B



2 / 10

FIG. 2A

*TCRBV5/BC*

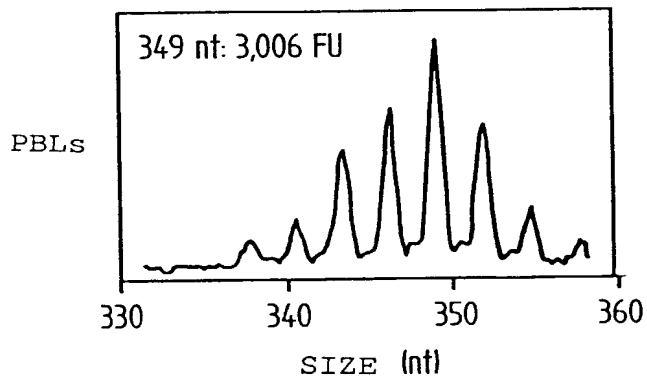
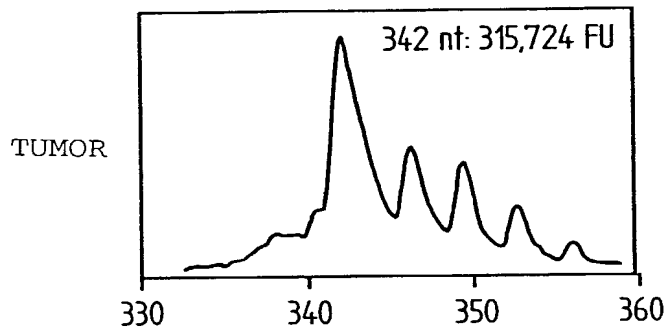
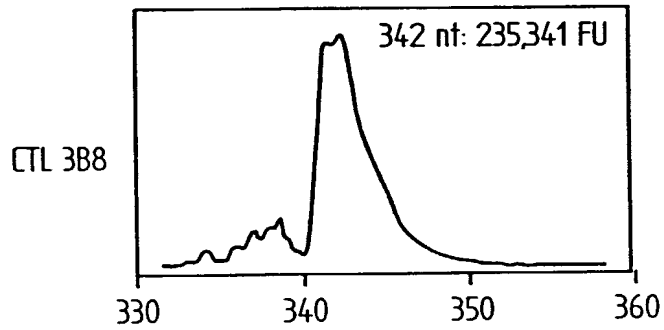
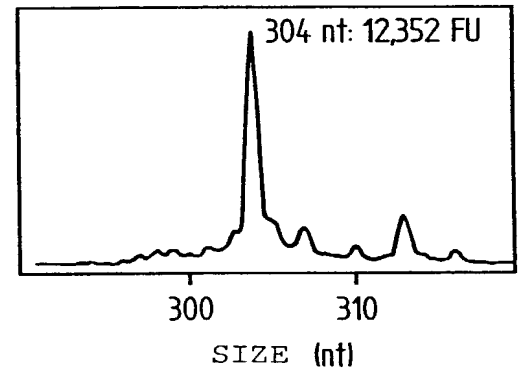
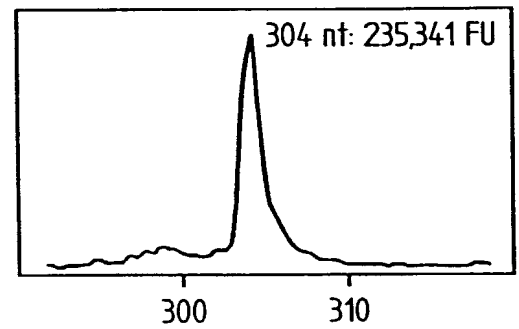


FIG. 2B

*TCRBV5/BJ1S2*



3 / 10

FIG.3

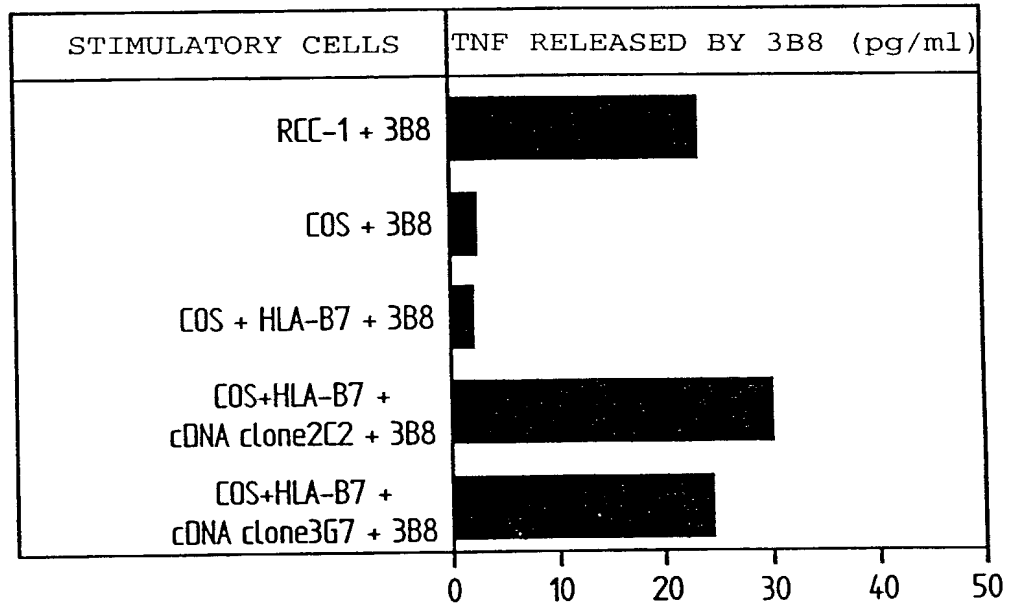
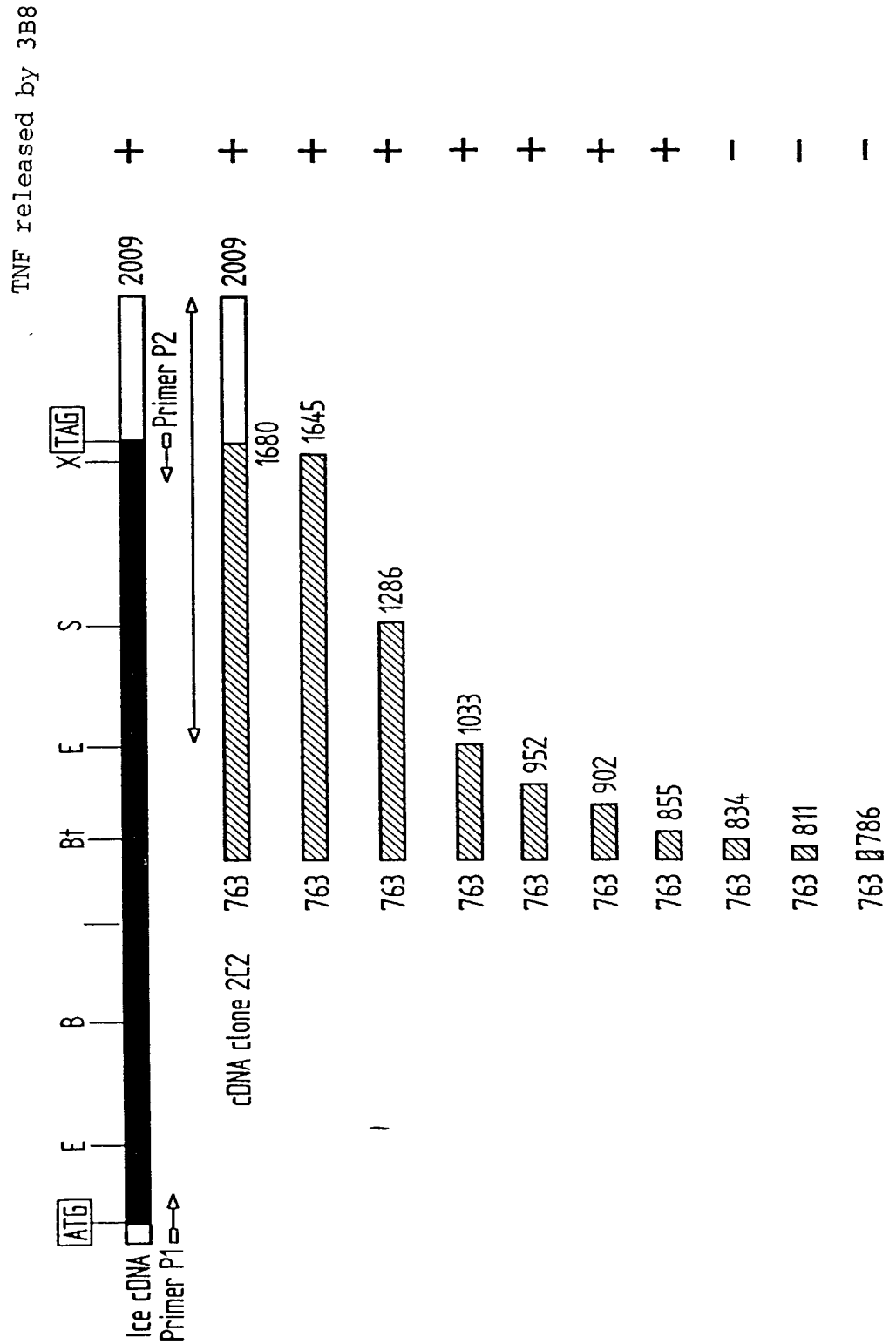




FIG. 4



5 / 10

FIG. 5

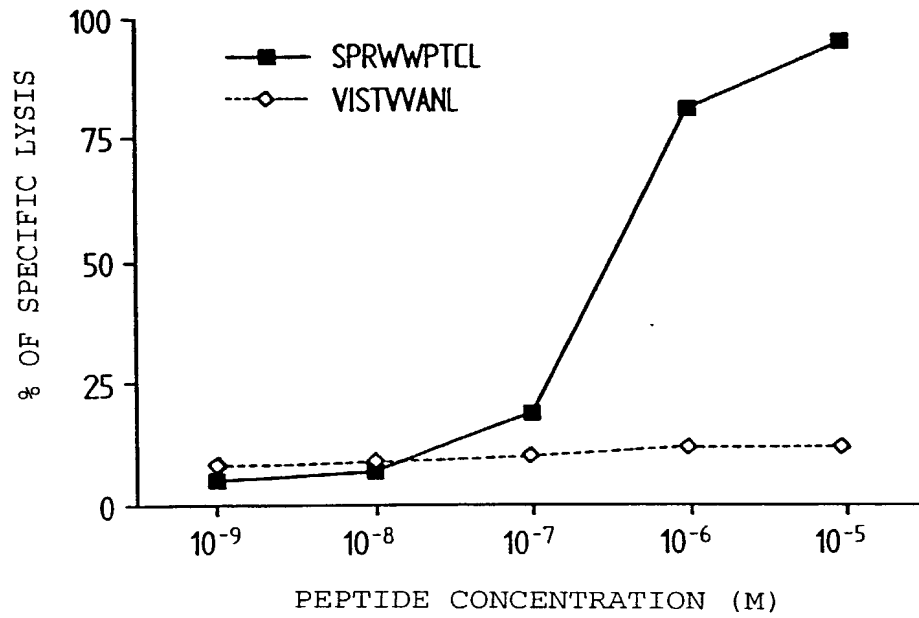
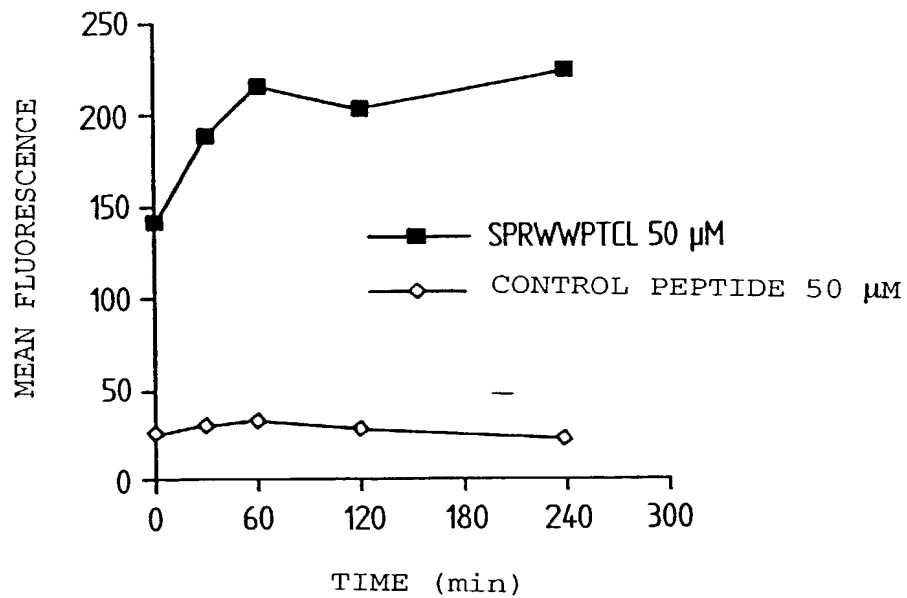


FIG. 6



6 / 10

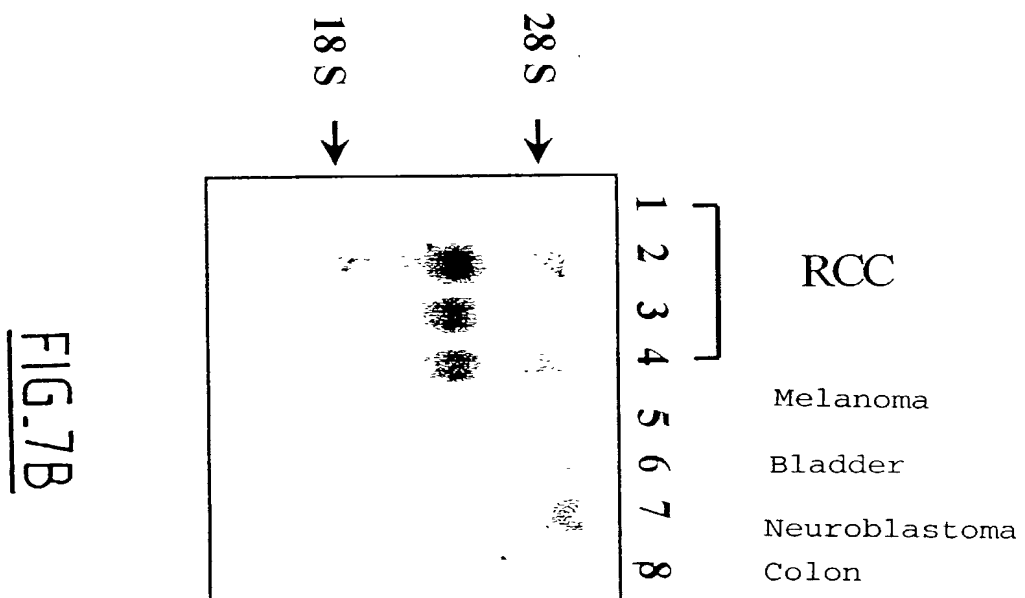
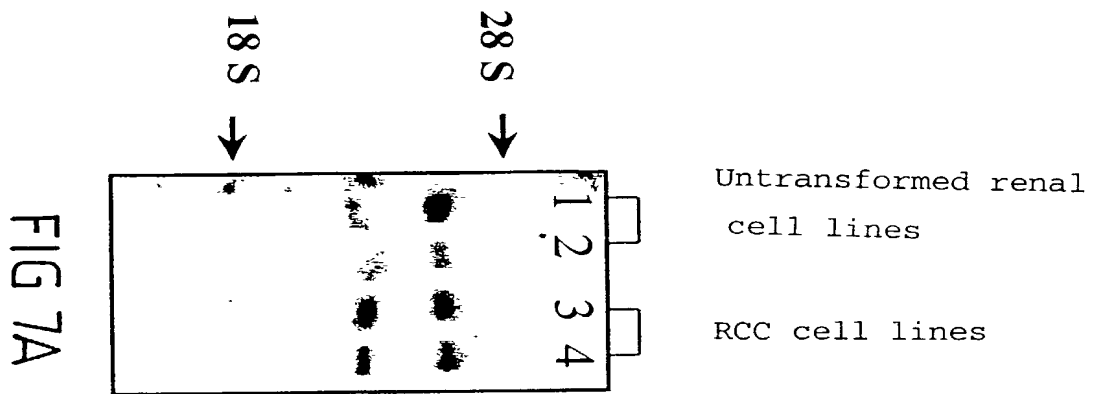


FIGURE 8A

**B** atGcggtgcacagacttcgtgcgggctgagcggtggcctgtggccttctgtgctt 60  
M R L H R L R A R L S A V A C G L L L L  
ctgtccggggccaggactcagccagtcacctcccgaccacacacacgggag  
120 L V R G Q G Q D S A S P I R T T H T G Q  
gtgctgggagtcctgtccatgtgaaggcgccaatgccgggtccaaccttcctggga  
180 V L G S L V H V K G A N A G V Q T F L G  
attccatttgccaagccacctctaggtccgctggtgattgcacccctgagccccctgaa  
240 I P F A K P P L G P L R F A P P E P E  
tcttgagtggtgtgagggatggaaccaccatccggccatgtgtctacaggacctcacc  
300 S W S G V R D G T T H P A M C L Q D L T  
gcagtgagtcagagttcttagccagttcaacatgaccttcccttccgactccatgtct  
360 A V E S E F L S Q F N M T F P S D S M S  
gaggactgcctgtacctcagcatctacacgcccgcctagccatgaaggctctaacctg  
420 E D C L Y L S I Y T P A H S H E G S N L  
**C**  
ccggtgatggtgtggatccacGgtggtgcgctgtttttggcatgGcttccttgtAtgAt 480  
P V M V W I H G G A L V F G M A S L Y D  
- W C G S T V V R L F L A W L P C M M  
ggttccatgctgctgccttggaacgtggtggtggtCatcatccagtagccgctgggt 540  
G S M L A A L E N V V V I I Q Y R L G  
V P C W L P W R T W W S S S T A W V

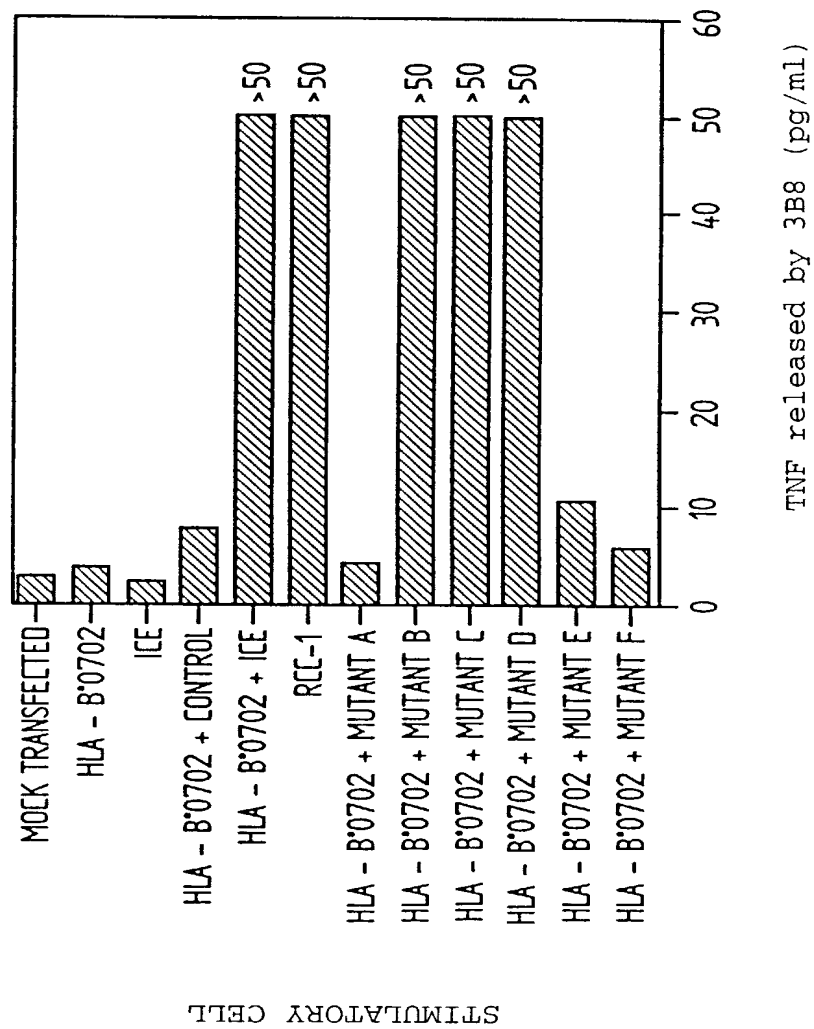
FIGURE 8A (continued)

600 gtcctgggcttcttcagcactggagacaagcacgcaacccggcaactggggctacctggac  
V L G F F S T G D K H A T G N W G Y L D  
S W A S S A L E T S T Q P A T G A T W T  
660 caagtggctgcactacgtgggtccagcagaatatcgccactttggaggcaaccctgac  
Q V A A L R W V Q Q N I A H F G G N P D  
K W L H Y A G S S R I S P T L E A T L T  
720 cgtgtCaccatttttggcgagtctgcgggtggcacgagtgtgtcttcgcttgtgtgtcc  
R V T I F G E S A G G T S V S L V V S  
V S P F L A S L R V A R V C L R L L C P  
780 ccataatccaaggactcttccacggagccatcatggagagtggcgtggccctcctgccc  
P I S Q G L F H G A I M E S G V A L L P  
P Y P K D S S T E P S W R V A W P S C P  
840 ggcctCattgccagctcagctgAtgtCatctccacgggtgggtggcaacctgtctgcctgt  
G L I A S S A D V I S T V V A N L S A C  
A S L P A Q L M S S P R W W P T C L P V  
900 gaccaagttgactctgagggccctgggtgggtgctgctgctgggggcaagagtaaagaggagatt  
D Q V D S E A L V G C L R G K S K E E I  
T K L T L R P W W A A C G A R V K R R F  
960 cttgcaattaacaagcctttcaagatgatccccggagtggtggatggggtcttctgccc  
L A I N K P F K M I P G V V D G V F L P  
L Q L T S L S R -  
1020 aggaacccccaggagctgtgctggcctctgcccgaactttcagcctgtccctagcattgttgggt  
R H P Q E L L A S A D F Q P V P S I V G

FIGURE 8A (continued)

gtcaacaacaatcggtcggtcgtcatcccccaagggtcatgaggatctatgataccag 1081  
V N N E F G W L I P K V M R I Y D T Q  
aaggaatggacagagagggcctcccaggctgctctgcagaaaaatgttaacgctgctgatg 1140  
K E M D R E A S Q A A L Q K M L T L L M  
ttgcctcctacatttggtgacctgctgaggaggaggtacattggggacaatggggatccc 1200  
L P P T F G D L L R E E Y I G D N G D P  
cagacctccaagcgagttccaggagatgatggcggactccatgtttgtgatccctgca 1260  
Q T L Q A Q F Q E M M A D S M F V I P A  
ctccaagtagcacattttcagtggttcccggggccctgtgtacttctacgagttccagcat 1320  
L Q V A H F Q C S R A P V Y F Y E F Q H  
cagccqagctggctcaagaacatcagggccaccgcacatgaaggcagaccatggtgatgag 1380  
Q P S W L K N I R P P H M K A D H G D E  
cttccttttgttttcagaagtttcttgggggcaactacattaaattcactgaggaagag 1440  
L P F V F R S F F G G N Y I K F T E E E  
gagcagctaagcaggaagatgatgaagtactggggccaaactttgcgagaaaatgggaacccc 1500  
E Q L S R K M M K Y W A N F A R N G N P  
aatggcgaggtctgccacactggccgctgttcgaccaggaggagcaataacctgcagctg 1560  
N G E G L P H W P L F D Q E E Q Y L Q L  
aacctacagcctgcggtggcggtgtctgaaggcccacaggctccagttctggaagaag 1620  
N L Q P A V G R A L K A H R L Q F W K K  
gcgctgccccaaaagatccaggagctcgaggagcctgaagagagacacacagagctgtag 1740  
A L P Q K I Q E L E E P E E R H T E L -

**FIG. 8B**



## DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

**PEPTIDE COMPOUND DERIVED FROM A SHIFTED ORF OF THE ICE GENE**

the specification of which is attached hereto unless the following box is checked:

☒ was filed on June 27, 2000 as United States Application Number or PCT International Application Number PCT/FR00/01791 and was amended on \_\_\_\_\_ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is known by me to be material to patentability as defined in Title 37, Code of Federal Regulations § 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed:

## PRIOR FOREIGN APPLICATION(S)

NUMBER	COUNTRY	DAY/MONTH/YEAR FILED	PRIORITY CLAIMED
99 08224	France	28.06.1999	yes

I hereby claim the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below.

APPLICATION NO.	FILING DATE

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s), or § 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is known by me to be material to patentability as defined in Title 37, Code of Federal Regulations § 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

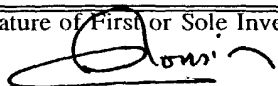
APPLICATION SERIAL NO.	FILING DATE	STATUS: PATENTED, PENDING, ABANDONED
PCT/FR00/01791	27.06.2000	pending

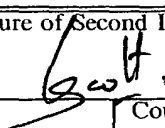
I hereby appoint as my attorneys, with full powers of substitution and revocation, to prosecute this application and transact all business in the Patent and Trademark Office connected therewith: Stephen A. Bent, Reg. No. 29,768; David A. Blumenthal, Reg. No. 26,257; William T. Ellis, Reg. No. 26,874; John J. Feldhaus, Reg. No. 28,822; Patricia D. Granados, Reg. No. 33,683; John P. Isacson, Reg. No. 33,715; Donald D. Jeffery, Reg. No. 19,980; Eugene M. Lee, Reg. No. 32,039; Richard Linn, Reg. No. 25,144; Peter G. Mack, Reg. No. 26,001; Brian J. McNamara, Reg. No. 32,789; Sybil Meloy, Reg. No. 22,749; George E. Quillin, Reg. No. 32,792; Colin G. Sandercock, Reg. No. 31,298; Bernhard D. Saxe, Reg. No. 28,665; Charles F. Schill, Reg. No. 27,590; Richard L. Schwaab, Reg. No. 25,479; Arthur Schwartz, Reg. No. 22,115; Harold C. Wegner, Reg. No. 25,258.

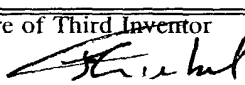


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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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Residence Address <del>XXXXXX de XXXXX Boulevard XXXXXXXX 94160 XXXXX de XXXXX de XXXXX</del> <b>NEW ADDRESS: 97 avenue Marguerite Renaudin, 92140 Clamart / France</b>	Country of Citizenship <b>France</b>	
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Full Name of Second Inventor <b>SCOTT Véronique</b>	Signature of Second Inventor 	Date <b>Dec. 12, 2001</b>
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Full Name of Third Inventor <b>TRIEBEL Frédéric</b>	Signature of Third Inventor 	Date <b>Dec. 12, 2001</b>
Residence Address <b>10 rue Saint-Louis - 78000 Versailles / France</b>	Country of Citizenship <b>France</b>	
Post Office Address <b>The same as residence</b>		

Full Name of Fourth Inventor	Signature of Fourth Inventor	Date
Residence Address	Country of Citizenship	
Post Office Address		

Full Name of Fifth Inventor	Signature of Fifth Inventor	Date
Residence Address	Country of Citizenship	
Post Office Address		

- 1 -

SEQUENCE LISTING

<110> INSTITUT GUSTAVE ROUSSY

<120> PEPTIDE COMPOUND DERIVED FROM A SHIFTED ORF OF THE ice GENE

<130> D18280

<160> 5

<170> PatentIn Vers. 2.0

<210> 1

<211> 162

<212> PRT

<213> Homo sapiens

<220>

<223> Polypeptide encoded by the (+1) alternative  
and shifted open reading frame of human ice

<400> 1

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Cys	Trp	Leu	Pro	Trp	Arg	Thr	Trp	Trp	Trp	Ser	Ser	Ser	Ser	Thr	Ala	20	25	30	
Trp	Val	Ser	Trp	Ala	Ser	Ser	Ala	Leu	Glu	Thr	Ser	Thr	Gln	Pro	Ala	35	40	45	
Thr	Gly	Ala	Thr	Trp	Thr	Lys	Trp	Leu	His	Tyr	Ala	Gly	Ser	Ser	Arg	50	55	60	
Ile	Ser	Pro	Thr	Leu	Glu	Ala	Thr	Leu	Thr	Val	Ser	Pro	Phe	Leu	Ala	65	70	75	80
Ser	Leu	Arg	Val	Ala	Arg	Val	Cys	Leu	Arg	Leu	Leu	Cys	Pro	Pro	Tyr	85	90	95	
Pro	Lys	Asp	Ser	Ser	Thr	Glu	Pro	Ser	Trp	Arg	Val	Ala	Trp	Pro	Ser	100	105	110	
Cys	Pro	Ala	Ser	Leu	Pro	Ala	Gln	Leu	Met	Ser	Ser	Pro	Arg	Trp	Trp	115	120	125	
Pro	Thr	Cys	Leu	Pro	Val	Thr	Lys	Leu	Thr	Leu	Arg	Pro	Trp	Trp	Ala	130	135	140	
Ala	Cys	Gly	Ala	Arg	Val	Lys	Arg	Arg	Phe	Leu	Gln	Leu	Thr	Ser	Leu	145	150	155	160
Ser	Arg																		

- 2 -

<210> 2

<211> 9

<212> PRT

<213> Homo sapiens

<220>

<223> Peptide fragment originating from SEQ ID No. 1  
and causing a specific T response

<400> 2

Ser Pro Arg Trp Trp Pro Thr Cys Leu  
1 5

<210> 3

<211> 521

<212> DNA

<213> Homo sapiens

<220>

<223> (+1) alternative and shifted open reading frame of iCE

<400> 3

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ctggagacaa gcacgcaacc ggcaactggg gtaacctgga ccaagtggct gcactacgct 180
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<210> 4

<211> 30

<212> DNA

<213> Homo sapiens

<220>

<223> Primer P1

<220>

<223> Sense

<400> 4

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<210> 5

<211> 30

<212> DNA

<213> Homo sapiens

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<223> Primer P2

